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THE GENERAL COURSE OF EVOLUTION IN THE PLANT KINGDOM.*

STUDIES IN DETERMINATE EVOLUTION, I.

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A study of the system of plant relationships inevitably leads to a consideration of the processes through which the system came into existence. Having spent many years in an attempt to learn something of the true phyletic relationships of plants, the writer desires to present, in a series of short papers, some of the remarkable phenomena which have come to light and which apparently have never been published heretofore in any adequate manner. These papers will bear the subtitle, "Studies in Determinate Evolution," so that they can easily be recognized as forming a part of a general presentation. The term, "Determinate Evolution" is used advisedly as expressing the actual condition of things in all evolutionary movements of a fundamental nature. All such primary movements attain a definite limit, sooner or later, beyond which no further movement in the given direction is possible. This broad generalization will be justified when some of the more important evidence has been submitted. All of the more fundamental taxonomic forms, therefore, fall into what will be called orthogenetic series, and these series are the result of progressive and perfective movements, which commonly are profoundly consistent in their development.

In general, evolutionary movements are either segregative or progressive. But the segregative mutations are determinative as well as the progressive mutations. Each segregative

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movement produces narrower limits within which further mutations are confined. Thus when class, order, family, or genus limits are once established all further movements are within the limits thus set up. It is this fact, together with the general principal of irreversibility, that is responsible for the taxonomic system of larger and smaller phyla. Furthermore, the different movements which may take place in an organism are largely independent of each other. There is a certain correlation of a general sort, nevertheless fundamental evolutions, as for example that of the flower, are not at all dependent on any special type of structure or function, nor on any definite degree of advancement in the series, nor on any special geological period or climatic cycle. The property or potentiality responsible for the development of flowers appears independently a large number of times at entirely different levels. Another important development which will make this principle evident is the evolution of the time of sex determination from the bisporangiate flower condition to the monecious and diecious conditions. This movement has taken place independently a very great number of times in the most diverse morphological systems and at every level of advancement from the very low gymnosperms to the very highest dicotyls. The more primitive movement of sex evolution in the gametophyte has proceeded in the same manner. The two evolutionary movements given above are characteristic of a very large number of cases which it is the intention to present as occasion permits.

As intimated, there is no correspondence between the general evolutionary movements and the environment. Evolution, from the beginning, has ploughed its way consistently through the environment. Evolution is kinetic and not static. It is progressive and orthogenetic; it is perfective and, as stated above, determinative. The movements in general have no special utilitarian import. The two older prominent hypotheses of evolution, namely Lamarck's utilitarian explanation on the basis of desires and of use and disuse, and Darwin's utilitarian selection are purely static conceptions and do not at all correspond to the actual systems of taxonomy which they are supposed to explain. Utilitarian and selective theories are anarchistic, depending on the circumstances or accident of the environment to bring about a movement. They are reasoning in a circle and a contradiction of the taxonomic system as it is actually evolved. If either Lamarckism or Darwinism were

true, we would have a fundamentally different taxonomic system and this system would be in harmony with the environment but would nevertheless be anarchistic if considered by itself, since the habitat itself is continually changing. If the changing habitat were the cause either of a direct utilitarian, evolutionary response or of a selective value, the organisms which had developed in it at a given time or had taken refuge in it in the first place would be subject to these cyclic changes and an anarchistic system would be the result. But nothing of the kind is present in the plant world. On the contrary there is a most remarkable cosmos. These older evolutionary hypotheses belong to the kindergarten period of causative biological science and are purely teleological in their implications. Evolution goes on without any reference to special teleological ends, except that we can say that the system developed has a general utility and fits into the environment in which it has taken place to a greater or less degree. Philosophically there is no contradiction but rather a confirmation of the principle of universal design. It is the principle of special design, or special teleological causation, that finds no basis of support in the taxonomic system. There is a general utility and a universal design plainly in evidence. The whole system does work admirably and has been successful for millions of years. But this universal principle of utility is something very different from the notions of special advantages and special utilities on which the older static theories are developed. Since any organism cannot be dissociated from an environment of some kind, the principle of general utility is always in evidence. But the same orthogenetic movements have taken place in environments diametrically opposite in nature, as for example, many exactly parallel movements of angiosperms into the water, on the one hand, and into mesophytic and xerophytic conditions, on the other. In the angiosperms as well as in many other groups, there is a profusion of parallelisms, yet since evolution is determinate, a species or a larger group of any kind when once lost can never be replaced or duplicated. This is the conclusive testimony of paleontology.

Leaving the consideration of the taxonomic system and its non-correspondence with environment, we find, nevertheless, that there is an abundance of ecological adaptation in details of form and function, but these ecological characters, in general, do not run parallel with the taxonomic system. And since the

taxonomic movements are known to be independent of specific environments, the ecological adaptations, because of a lack of evidence to the contrary, must be considered to be brought about in the same general way, namely, through intrinsic changes which take place in many environments but which are occasionally of such a nature that the environment exercises a decisive eliminative effect. This must be the scientific inference in regard to ecological adaptations until acquired characters can be shown to be induced in the phylogenetic line as readily as they are induced in the ontogentic cycle. Instead of having natural selection in the old sense of the term, we have natural or selective elimination. But this elimination is to be considered as operative only in very special physiological and morphological developments. The vast majority of forms, structures, and functions have an agreeable survival value in the same general environment. The same general environment, for example, will harbor members of the plant kingdom from one end of the series to the other as well as a miscellaneous assemblage of animals. Furthermore, structures of exactly opposite nature in homologous parts survive in the same environment, and sometimes these structures may have a fanciful utility and sometimes not. As stated before, evolution has been described as irreversible and this is true for all fundamental movements. It is only in the extremely minor details that reversibility seems possible and this, as the paleontological record shows, has never involved any of the general movements of the organism, whether plant or animal. It must be emphasized that, in a general sense, evolution is non-selective and non-utilitarian. Very frequently a utility evolves that is of absolutely no value to the possessor, when compared with a lower, related form that has not evolved the utility; and since evolution is orthogenetic and determinative, there are frequently also developments which pass far beyond the limits of practical utility to the realm of the so-called over-adaptations. In some cases also, the development is a positive disadvantage to the possessor.

Evolution and the taxonomic system which comes from it are, therefore, anything but anarchistic systems. Those who hold to an anarchistic system of philosophy, whether political or scientific, need not look for support of their views from a study of the phyletic taxonomic system found in the plant kingdom.

The first part of the problem of evolution is to find out exactly what the process is and what has been the result of its operation, and after we have discovered some of these things we may proceed to investigate the causes of the process. In the past, evolutionary science so-called has commonly proceeded in the opposite direction. A dogmatic hypothesis was formulated, based on some preconceived philosophical attitude and then isolated facts were assembled which appeared to give a plausable proof of the assumption. This procedure is not science and has in the past resulted in enormous reversals and repudiation of widely accepted theories. True inducive science demands that theory shall follow a certain foundation of ascertained phenomena. It also demands that there be no confusion of fact and assumption and that reasoning in a circle shall be tabood.

THE EVOLUTION OF THE GENERAL SERIES.

Without going into the question of the origin of the protoplast, which would take us too far afield, we will begin with a consideration of the general nature of the lowest known organisms. These organisms have a definite protoplast or a definite unitary cell organization. Whatever its origin, the cell is established as the unit of the living reaction system. Life phenomena are manifested by cells. Thus the organized cell is the starting point and the basis on which a complicated reaction system is built up, proceeding step by step from the lowest to the highest stage attained in the evolutions of organisms. The building up or evolving of the fundamental reaction system has been attained by adding at each stage some new potentiality or potentialities which were not there before. The new fundamental properties are laid down on top of the old. The old is not destroyed nor even inhibited but the new simply makes the reaction system more complex. This general evolution is, therefore, also to be regarded as an orthogenetic progression, although this may not be so manifest at first thought as some of the remarkable movements which take place in some of the greater or smaller phyla. When one remembers, however, that these new properties are commonly introduced not simply in one line but into all the advancing lines, then their evolution becomes the most profoundly remarkable of all the serial, orthogenetic movements known.

In the lowest plants, the cell is not nearly so elaborately organized as it becomes a little farther up the scale. These lowest plants are properly called PROTOPHYTA and the very lowest are apparently the group of the *holophytic bacteria*. There is no sex potentiality but the general properties manifest are: (1) unitary nature and activity of the protoplast, (2) assimilative property or hereditary perpetuation of the system, (3) division of the protoplasts or cells, and (4) separation of the units after division. These properties are present in all higher organisms also but the separation of cells becomes greatly restricted. They are the first and fundamental potentialities of all living things whether plant or animal. There are a number of important physiological systems established which are of a segregative character as various types of metabolism resulting in holophytic, saprophytic and parasitic groups, flagellate and non-flagellate, chlorophyll-bearing and chlorophylless groups, etc. This subkingdom, therefore, represents in itself a great period of evolution.

THE FIRST GREAT TRANSITION.

In passing from the lowest to the higher organisms, both animal and plant, we find that there are three fundamental and universal advances. (1) The development of the colony or multicellular condition, brought about by a delay and usually a restriction of the process of cell separation after division; (2) The development of the process of differentiation of cells and tissues in the body, and (3) the development of the sex-potentiality. For purposes of a definite taxonomy it becomes necessary to choose one of these three—multicellular condition, differentiation process, or sexuality—as a basis of segregation, since the appearance of the three characteristics are not coincident in the various primitive groups. The most suitable and definitely determinable characteristic is the sex-potentiality. Therefore, the Protophyta are made to include all the plants which are nonsexual as contrasted with those having sexuality, namely the METAPHYTA. As stated, some Protophyta are multicellular and differentiated while some of the lowest Metaphyta are unicellular or are undifferentiated. However, the evolution soon proceeds far enough that shortly all organisms, whether plant or animal, possess the three added characteristics, namely, multicellular condition, differentiation,

and sexuality. All the higher Metaphyta and all the Metazoa therefore possess the seven characteristics mentioned thus far.

With the evolution of the sex-potentiality there are possible, from time to time, three general states in the cell lineage: (1) the female state, (2) the neutral state, and (3) the male state. In some plants the neutral state is the prevailing condition in others the neutral condition may be rare or never properly developed. With the advent of sexuality also there is an entirely new mode of hereditary transmission. The two fundamental features are primary sexualization of the haploid gametes, resulting in fertilization, and primary sexualization of the chromosome sets in the diploid reproductive phase, resulting in the reduction division. These processes are responsible for Mendelian segregation of heredity and Mendelian interaction of dominant and recessive allelomorphs in the diploid cell. These new phenomena are also characteristic of all the higher plants and animals.

PROGRESS IN THE SECOND SUBKINGDOM.

The NEMATOPHYTA show the progressive acquirement of a number of very important general properties among which the most important are: (1) the attainment of dimorphic gametes, and (2) the evolution of secondary sexual states and characters. It is needless to say that these advances are universal when once attained and are shared also by the animals. The appearance of secondary sexual characters is brought about by a shifting back of the time of sex determination into an earlier stage of the ontogeny before gametogenesis. These secondary sexual states give rise to dimorphisms by influencing hereditary expression but the cells do not manifest the attractive properties which appear later in the gametes. Thus the higher plants have a five-fold manifestation of the sex-potentiality in the cell lineage: (1) neutral state, (2) secondary female state, (3) primary female state, (4) secondary male state, (5) primary male state.

TRANSITION TO THE METATHALLOPHYTA.

The transition from the THALLOPHYTA to the METATHALLOPHYTA represents the greatest break or hiatus in the plant series. The Metathallophyta are a monophylletic group when compared with the lower forms. There was a long period of

evolutionary advancement in which the ancestors of the group developed all the fundamental characteristics outlined above for the Thallophyta and in addition the Metathallophyta evolved a characteristic life cycle. All of the higher plants have a uniform type of life cycle namely, the typical, antithetic alternation of generations with a haploid gametophyte and a diploid parasitic sporophyte. In this cycle there are twelve characteristic stages. These stages are retained to the end of the plant series. In the Thallophyta there are several types of alternation of generations but none that corresponds to the typical antithetic cycle of the higher plants. In most cases the sporophyte is an independent plant, like the gametophyte. The nearest approach is in the red algæ where the zygote undergoes vegetative growth and produces a number of carpospores which are discharged and develop the independent sporophyte. The twelve stages of the typical antithetic alternation of generations cycle are as follows: (1) Haploid gametophyte, (2) dimorphic gametangia, (3) dimorphic gametes, (4) fertilization, (5) oospore or zygote, (6) germination in the ovary, (7) diploid sporophyte, completely parasitic or parasitic in its embryonic stage only; (8) sporangium, (9) sporocyte, (10) reduction division, (11) tetraspores, (12) germination.

BRYOPHYTE STAGE.

This transition leads up to the third subkingdom, or general evolutionary stage, called the BRYOPHYTA. The parasitism of the sporophyte is complete. The sporophyte is also completely determinate in its growth, since the production of its spores either involves the entire individual or its single terminal growing axis. Only in one group is there a peculiar type of intercalated indeterminate growth evolved and this is usually of short duration. There is a marked progression in the size and importance of the sporophyte as compared with the gametophyte, and in general this forward movement in the evolution of the sporophyte persists to the very summit of the plant kingdom. The sporophyte is entirely neutral, the sex potentiality never giving rise to sexual states in the normal conditions. The lowest species have a sporophyte that is nothing but a sporangium, representing a hollow sack of spores; the highest species have a fairly well organized body with foot, stalk,

photosynthetic organ with stomata (hypophysis), and sporangium, and in the Anthocerotæ, as intimated above, even a crude beginning of indeterminate growth. One of the most important progressions is in the continually smaller area of tissue involved in the spore reproductive process. In the lowest species the entire individual except the epidermal layer is transformed into spores; in the higher liverworts there is sterile tissue interspersed with the spores in the sporangium; while in the very highest the central part of the axis remains vegetative, thus shifting the reproductive process to the outer regions of the stem. This is of most profound significance from an evolutionary point of view as will appear below.

TRANSITION TO THE VASCULAR PLANTS.

The transition to the vascular plants is accomplished by the addition of heredity which causes a shifting of the reproductive process of the sporophyte from the terminal bud (cauline sporangia) over to the newly evolved lateral appendages of the stem, or the leaves, thus giving rise to sporophylls which with some modifications are characteristic of all vascular plants. The growing axis is now an indeterminate system. Thus the transition from bryophyte to homosporous pteridophyte is a change from a determinate sporophyte to an indeterminate sporophyte, and this leads over to a two-phased condition, the first phase of the sporophyte being parasitic and the later phase entirely independent. This two-phased life of the sporophyte, thus evolved, is characteristic of all higher plants. It is again a fundamental characteristic imposed on the one-phased condition of the bryophyte type of sporophyte. Of course, the whole morphological condition advances in complexity by the appearance in the sporophyte of potentialities for the development of a vascular system, leaves and roots. As stated above, the shifting of the point of spore production from the stem axis to a lateral appendage of the stem is not a sudden advance but shows in itself a definite orthogenetic advancement. It is evident that this shifting of the reproductive center from the region of the stem axis was well advanced before the appearance of a definite vascular system, so that with the appearance of lateral appendages the final step to sporophylly was but the culmination of a movement begun far down in the bryophyte stage.

Whether the lowest vascular plants originally produced nothing but sporophylls or whether from the beginning they developed alternating zones of foliage leaves and sporophylls is of no great consequence, and can probably never be proven one way or the other. Our living primitive vascular plants apparently all have such alternating zones. One thing is certain. The vascular plants did not originate from a strobilus-like sporophytic ancestor. The flower is a secondary determinate system evolved later from or in the earlier indeterminate system. The shifting of the point of spore production from the terminal bud to the lateral stem appendages was the primary condition for the development of indeterminateness. Now in the higher plants the lateral shifting of the reproductive function progresses still farther to the lateral organs of secondary axes as in cycads like *Macrozamia* where the cones are always lateral organs. In *Equisetum* and many conifers like spruces, firs, pines, etc., the terminal buds of all primary axes remain entirely vegetative and the flowers are developed on secondary or tertiary axes or on axes still more remote from the primary.

IMPORTANT ACQUISITIONS IN THE STAGE OF THE PTERIDOPHYTA HOMOSPORÆ.

Among the progressive movements developed in the homosporous pteridophytes is the evolution of dimorphism between foliage leaf and sporophyll. This condition is established in this fourth subkingdom and becomes more intense or extreme, in general, the farther one proceeds along the line of any of the higher phyla. The development of the flower or determinate, sporophyll-bearing axis, is also attained in some lines of homosporous pteridophytes and becomes characteristic and more pronounced and extreme as one reaches the highest phyla. The advancement in this respect is extremely definite and extremely orthogenetic. As in many of the bryophytes, some of the highest species have also shifted the time of sex determination in the gametophyte back in the ontogeny to the spore itself so that the gametophytes become unisexual.

TRANSITION TO THE HETEROSPOROUS PLANTS.

The transition between the fourth and fifth subkingdoms is again a fundamental one involving a definite condition of sexuality. It was just as important for the origin of the

peculiarities of the higher plants as the origin of sex itself was at the first transition in giving rise to a new type of hereditary phenomenon. In this transition a new potentiality is introduced which causes the time of sex determination to be shifted backward in the life cycle from the beginning of the gametophyte to the end of the sporophyte, from which point it proceeds in many groups back to the beginning of the sporophyte. This step was probably necessary for the evolution of any kind of seed habit. All the main lines of pteridophytes and, of course, all the lines that evolved the seed habit later took this extraordinary evolutionary step. It was a new potentiality which determines functional states or gradients of a certain type in the diploid sporophyte and these in turn cause secondary sexual states to arise. In the sporophytes of bryophytes and homosporous pteridophytes no such states appear normally although the cells have the sex potentiality. Thus the new heredity gives rise to trimorphisms and dimorphisms in the sporophyte, while as stated all sporophytes below, whether bryophytes or homosporous pteridophytes are normally homomorphic in respect to sex because they remain in a neutral state. The secondary sexual states which arise determine the character of the reduction spores and thus the sexual states of the gametophytes are pre-determined. All gametophytes of heterosporous plants are unisexual while those of the homosporous, whether bryophytes or pteridophytes, are more commonly hermaphroditic. Along with the development of secondary sexual states in the reduction spores goes the great reduction in size and importance of the gametophyte generation, especially the male gametophyte. This latter condition paves the way for the evolution to the seed habit. The orthogenetic and progressive character of this great movement, as well as the intrinsic nature of the evolutionary process, are clearly in evidence when one considers the numerous independent appearances of the condition.

TRANSITION TO THE SEED PLANTS.

As stated, the development of PTERIDOPHYTA HETEROSPORÆ, through the shifting of the time of sex determination, was a necessary step for the development of the fifth transition. This evolution adds a number of fundamental properties to the system, and a large number of almost simultaneous move-

ments take place which make the history of the transition to the seed plant condition appear almost like a fairy tale. The great central movement in this transition was the retention in the mature sporangium of the spores giving rise to the development of the parasitism of the gametophytes. This condition imposed upon the fundamental complex of properties already attained was a truly marvelous step in advance. The reduction spores are now in the same evolutionary stage as the zygote was from the liverworts on up. The female gametophyte is permanently parasitic and enclosed in the megasporangium (ovule). Thus the development of the parasitic relation between the two generations has reached the final limit possible. The telescoping has advanced as far as the conditions of life will permit. The orthogenetic movement of one generation depending on the other has reached its ultimate possibility. It is as though a short snake swallowed a long snake's tail and then the long snake turned around and swallowed the short snake along with its own tail. The seed of the GYMNOSPERMÆ is thus a triple organism representing two generations and a part of a third. The male gametophyte acquires a double-phased life. At first it is parasitic in the microsporangium and later it is parasitic in the ovule through the development of the pollen-tube. This two-phased condition is again carried through the last subkingdom, the ANGIOSPERMÆ. A remarkable addition is also made to the previous complement of fundamental potentialities of the sporophyte. The sporophyte is still a two-phased individual but with the intercalation of a resting condition or a greater or less degree of dormancy between the two phases. Several additional minor details of complexity are added at the fifth evolutionary transition to the hereditary equipment of the plant which need not be mentioned here. One however is very interesting. The parasitic sporophyte individual in the seed, which becomes the plant to struggle with the outer world is now protected to the extreme and supplied with food for use after its reawakening, by means of which it can establish itself promptly as an independent organism when opportunity is afforded. The higher plants no less than practically all the higher animals have taken their offspring out of the struggle for existence with the external world, and have provided a most advantageous condition of opportunity for survival and also for a favorable migration from the parent habitat. At the level of seed evolution nearly all phyletic

lines have also attained to the complexity of determinate reproductive shoots or flowers, only two of the lowest living genera of gymnosperms being flowerless, namely, the carpellate plant of the species of *Cycas* and both carpellate and staminate plants of *Ginkgo*. The acquisition of the flower is another striking example of multiple progressive movements attained in all but a few of the main lines of vascular plants. When once attained also the flower has never devoluted, although in the lower types proliferation, as one would expect from the establishment of any such fundamental potentiality, is common.

TRANSITION TO THE ANGIOSPERMÆ.

The sixth transition leads to the highest subkingdom, the present culmination, and probably the final one, in the fundamental progressive evolution of the plant kingdom. The important change is the closing of the megasporophyll around the ovules which necessitates the acquisition of a new organ of pollination, the stigma. The closing of the sporophyll was accomplished in some lines of ferns but, of course, no stigma is present in these. The presence of a stigma and closed ovulary in the carpel requires an additional development of the pollen-tube. The pollengrain now germinates, not in the micropyle of the ovule as heretofore, but on the stigma. Thus the pollen-tube has an extensive growth before it enters the micropyle or some other part of the ovule. The angiosperms also attain the limit in many lines of determinate, floral development in the epigynous condition and in the extreme development of zygomorphy. The first mentioned condition never being attained in any lower plants and the second only to a very slight degree.

Another, and really the most profound of the added potentialities in the evolution of the Angiospermæ is the process of triple (or sometimes multiple) fusion of two cells of the female gametophyte with a sperm to form the triploid, definitive nucleus from which a distinct generation, the endosperm or xeniophyte, develops. This process makes the life cycle and the genetics of the angiosperms much more complicated than those of any other organism, either plant or animal. The seed now contains structures representing four distinct generations, two diploid generations, a haploid generation, and a triploid or sometimes a polyploid generation.

THE ARRESTED LIMITS OF THE EVOLUTIONARY PROCESS.

There is another fundamental fact which stands out and demands solution which is just as remarkable as the evolutionary progression itself. Why did the various groups of plants remain on the several levels which they have attained? There is a principle of stability involved which makes living organisms the most enduring things that we know of. There is also no evidence that any of the members of any level will ever evolve beyond the general limits to which they have attained. There may be endless evolutions in a subordinate way but the whole taxonomic system and the whole paleontological history indicate that the general process has been accomplished. It is also very doubtful whether any very great advance could be possible out of the angiosperm condition which might give an eighth subkingdom of plants. All the fundamental possibilities, as complexity of life cycle, evolution of the time of sex determination, reproductive system, interactionary parasitism, limit of floral evolution, etc., seem to have reached the limit of possible fundamental advancement of the system. Paleontology and a study of the present taxonomic system is a sufficient answer to any such fantastic speculation. So all we can say at the present in answer as to why a bacterium never got beyond the nonsexual unicellular stage, why a *Platanus* or a *Sassafras* of the present day is the same as a *Platanus* or a *Sassafras* of the time of the beginning of the Cretaceous period, why a liverwort or a moss never got beyond the liverwort or moss condition while other things at the same time were advancing in a most remarkable manner, is that in all such cases something appeared in the system which apparently forever prohibited a mutative change in the given direction, forever inhibited the addition of the essential potentiality into the given protoplast. As stated, this problem is just as fundamental and demands just as much attention and investigation as the actual evolutionary advancement and change itself.

CONCLUSION.

Now anyone who will take the trouble to understand what the entire evolutionary process outlined above implies will readily see that we are dealing with a kinetic principle, with a principle of progressive and perfective movement, with a most

remarkable accumulative or building up process, with a system which proceeds in the same general way in many diverse morphological and physiological complexes, right through the diversity of environments which are now on the Earth or which have existed in past geological ages. It is also clearly evident to any one, who knows something about the taxonomic system of plants and the geological history of organisms, that the progressive movements, like the evolution of the flowers in the various phyla, for example, have not at all taken place at the same time but often in entirely different geological horizons. When one comprehends all of this, then it becomes evident that much of the speculation of the nature and cause of evolution, developed in the past and still held to a considerable extent in the present, are, to repeat the proposition stated in the introductory part of this paper, to be regarded as amateurish beliefs mostly supported by the common illogical devise of reasoning in a circle, from which biological science should have been freed long ago. In this category are to be placed such teleological hypotheses like Lamarack's use and disuse through the sudden acquirement of new desires, Darwin's natural selection, sexual selection, and selective mimicry, and especially all the teleological vagaries of the Neo-Darwinians, as well as the unsupported belief in a direct specific causal effect of environment and in the inheritance of acquired characters.

The general array of facts and phenomena presented in the outline of the general fundamental evolutionary movements thus indicate that: Evolution is intrinsic, kinetic, progressive, orthogenetic, perfective, and determinative.

DEMONSTRATION OF THE LIFTING POWER OF EVAPORATION.

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The lifting power of evaporation and the liquid tension present in water are two important forces in the transpiration stream. The desirability of having a better conception of these forces has led to numerous experiments. A modified Askenasy experiment has proven quite successful in demonstrating these forces in moving materials. A clearer picture can thus be impressed upon the minds of students in botany by actual demonstration.

An attempt was made first to perform the experiment using such materials as are usually available in a general botany laboratory and second to make the demonstration less cumbersome and more successful. The results obtained with the apparatus described in this paper have shown that with a relative degree of precaution, these two forces can be demonstrated in every laboratory.

Askenasy in 1896 fused a glass funnel on the upper end of a long glass tube. A layer of plaster of Paris was placed in the broad upper end of the funnel. After the plaster hardened the apparatus was filled with water and the end of the glass tube dipped in mercury. As the water evaporated from the plaster surface the mercury rose in the tube and attained a height of 82 cm. Ursprung (1913) used a porous porcelain cylinder as an evaporating surface. He improved considerably upon the technique and obtained much better results. However the demonstration was still quite unwieldy. A great improvement in the set up of the apparatus, as worked out by Lubin, is included in Livingston's 3rd edition of Palladin's Plant Physiology (1926).

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The apparatus used in these experiments was essentially the same as Lubin's arrangement, and with the few improvements made, is shown in the diagram (Fig. 1). The materials needed for the apparatus are: a porous porcelain cylinder or cup, a long J-shaped, thick walled, small bore glass tube, a bottle containing mercury, a large beaker, a source of heat such as a Bunsen burner, a vacuum pump and a one-hole and a two-hole rubber stopper.

In preparation for a demonstration, the apparatus was first thoroughly cleaned. The porous porcelain cylinder or cup (C) was filled with distilled water and attached by means of the one hole rubber stopper (D) to the short arm of the J-shaped vertical glass tube (E). The porcelain cylinder was then submerged in a beaker of water. The end of the long arm of the glass tube was fastened into the bottle containing the mercury by means of the two-hole rubber stopper (H) so that the end of the tube dipped below the surface of the mercury. The attachment for the suction pump (G) was fitted into the second hole of the rubber stopper.

After the apparatus had been assembled the water in the beaker was heated to boiling and by applying suction to the long arm of the J-shaped tube the hot water was caused to filter through the system. After the apparatus had been thoroughly washed and all undissolved air had been driven from the system by boiling it was allowed to cool to room temperature. By allowing the apparatus to stand, dissolved air could penetrate the entire system. Consequently tension was shown in water solutions containing dissolved air which no doubt is the condition of the water contained in the plant vessels and tissues. With the apparatus set in this manner, however, liquid tension could not be obtained. It was found necessary to substitute a colloidal coat for the evaporating surface of the porcelain cup. The coating used was a colloid such as agar agar or gelatin.

A colloid coat has two advantages: 1. A colloidal gel can be more easily duplicated than a porcelain surface and by coating several cylinders with the same colloidal gel, one can be sure of having relatively uniform evaporating surfaces on several cups. 2. Coating eliminates the difficulty encountered by former workers in finding porous porcelain cylinders that were sufficiently fine pored to support the air water menisci against several atmospheres of pressure. Some of the colloidal coats tend to

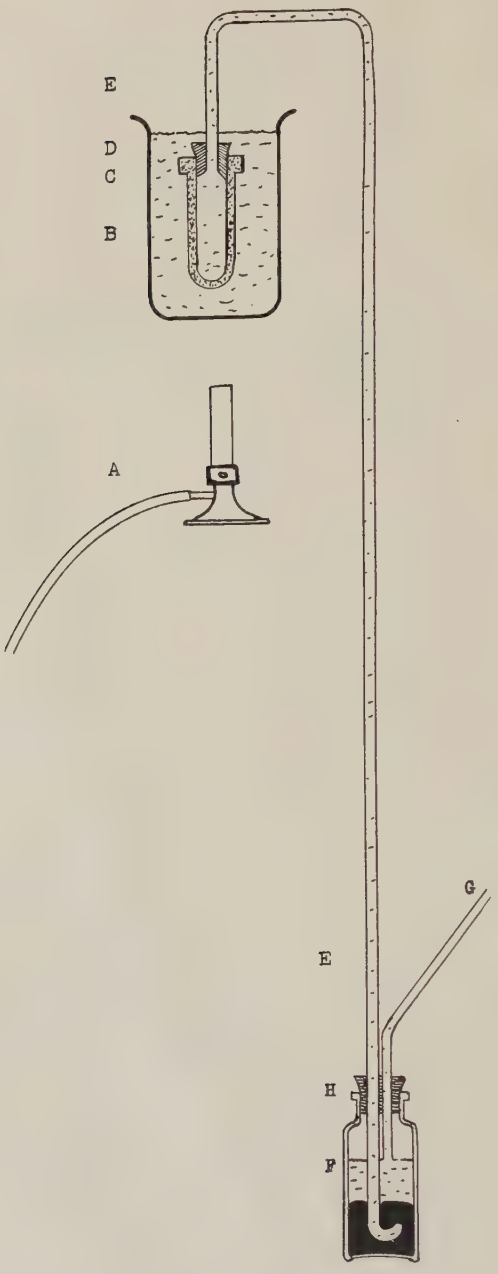

FIGURE 1

deteriorate or wash off when the cylinder is submerged in the beaker of water. A discussion of the characteristics of the various types of coats used will be included with the results.

The general method of applying the coat was as follows: the beaker was removed from around the porous cup and immediately 100 to 150 c.c. of the colloidal sol was poured over the rubber stopper and porous cup. Care was taken to see that the entire cup surface has been covered by the colloidal sol. A sufficient amount of the sol adhered to the cup to form an evaporation surface. When collodion (dissolved in alcohol and ether) was used as the colloidal sol it was found necessary to first wipe off all excess water from the surface of the cup.

The suction pump should be detached before allowing evaporation to take place. The evaporation of the water from the cup caused a decrease of water volume and this decrease of volume was compensated by the drawing of the mercury up into the glass tube. This process continued until a break occurred somewhere in the water or mercury column. Most of the breaks occurred in the water column. As soon as a break or bubble occurred the level of the mercury, if it was above atmospheric pressure, dropped to a level corresponding to nearly atmospheric pressure.

All that was necessary for a second trial was to submerge the porous cup in the beaker of water and the infiltrating water would force the bubble from the system. Applying suction to the long arm of the glass tube hastened the process. Cutting the lower surface of the one-hole rubber stopper so that it tapers toward the glass tube and bending the tip of the long arm of the glass tube, as shown in the diagram, often aided greatly in the removal of bubbles. After the bubble was removed, the suction pump was detached and the porous cup exposed to the air. Air currents from an electric fan hastened the evaporation process.

The time required to run a demonstration could be divided into two parts, first the cleaning and boiling of the apparatus and second the running of a trial. An hour of boiling the water in the beaker, simultaneously applying suction, has been found sufficient to remove all undissolved air. The apparatus was then allowed to cool to room temperature. The apparatus may be boiled a day or two previous to a trial, thus saving time. The time required for a trial after the coat has been

applied varied from ten minutes to several hours depending upon the bore of the glass tube, the consistency of the colloidal coat applied, the humidity and movement of the air and the height reached in the trial. Ten to twenty-five minutes were required to attain a height of 100 to 150 cm. in a one m.m. tube when a 20% gelatin coat was used.

The results or heights given have been corrected by the following formula:

$$H = h + c + \frac{W_1 - W_2}{13.6}$$

H = Total height of mercury column.
h = Ht. of mercury meniscus in tube above the mercury level in the bottle.
c = Correction for mercury depression in glass tube.
W_1 = Height of water column from the mercury meniscus to the top of the glass tube.
W_2 = Height of water standing above mercury in bottle.

By subtracting the atmospheric pressure from the total height (H) one has the height in terms of mercury that was supported by the cohesion or liquid tension of the water column.

Gelatin, agar agar, gum arabic, tragacanth, Karo syrup and collodion were used as evaporating surfaces. Sols of the first four were prepared by placing a definite number of grams of the dry granular substance in 100 c.c. of water and then heating to boiling in a water bath. These sols are referred to later as percentage sols. They were applied to the porous cups soon after being removed from the water bath and thus had a temperature of 90–98°C when applied. The Karo syrup and collodion were applied in the commercial form and at room temperature.

The following table gives some of the results. These results, excepting tragacanth, were obtained in glass tubing of 2 m.m. diameter. The trials were all made at room temperature 20 to 25°C.

Sol or Solution	Number of Trials	Average for Trials. Ht. in cm.	Highest Reading	
			Day of Trial	Ht. in cm.
20% Gelatin.....	41	129.1	Dec. 1	188.7
2% Agar Agar.....	22	120.4	Nov. 2	146.1
10% Gum Arabic.....	17	128.9	Nov.30	168.4
5% Tragacanth..... (1 m. m. tube)	3	120.5	Mar. 7	130.6
Karo Syrup.....	2	111.4	Dec. 6	128.1
Collodion.....	6	91.8	Oct. 5	99.2

The sols or solutions were arranged in the table according to their practicability as far as it was possible to determine. A gelatin, agar agar or collodian coat could be used repeatedly as these coats did not wash off when immersed in water. The height efficiency of the collodion, however, placed it at the bottom of the list. Gum arabic, tragacanth and Karo syrup could be used as quite efficient evaporating surfaces for single trials. These coats however wash off when the porous cups were immersed in water in attempting to remove air bubbles from the system.

Gelatin seemed to offer the best type of coat. The following table gives the results obtained from a good 20% gelatin coat.

Cup boiled Nov. 29 (1–3 P. M.)
Cup coated Nov. 29 (8 P. M.)

Time, 1927	Total Ht. cm.	Barometer Ht. cm.	Ht. above Barometer
Nov. 29—8– 9 P. M....	96.6 (Fan)	74	22.6
Nov. 30—2– 3 P. M....	184.4 "	73.5	110.9
Nov. 30—7– 9 P. M....	167.2 "	73.7	93.5
Dec. 1—3– 4 P. M....	188.7 "	75.3	113.4
Dec. 1—7– 9 P. M....	93.6 (No Fan)	75.3	18.3
Dec. 2—2– 4 P. M....	179.8 (Fan)	74.6	105.2
Dec. 3—8– 9 A. M....	144.3 "	75.3	69
Dec. 4—9–10 A. M....	136.5 "	75	61.5
Dec. 5—3– 4 P. M....	132.2 "	74.5	57.7

The four highest records obtained by using gelatin are given in the following table. Data corrected to 25°C.

Height Records.

Time, 1927	In 2 mm. Tube	Coat of 20% Gelatin	
	Total Ht. cm.	Barometer cm.	Ht. Above Barometer
Nov. 30.....	184.4	73.5	110.9
Dec. 1.....	188.7	75.3	113.4
Dec. 2.....	179.8	74.6	105.2
1928	In 1 mm. Tube	Coat of 25% Gelatin Sol	
Mar. 11.....	226.6	73.7	152.9

A gelatin coat tended to deteriorate but one coat was generally good for from five to ten trials, provided the trials were made within a week.

Summary.

The above experiments, with an improved Askenasy apparatus may be summarized as follows:

1. Liquid tension and the lifting power of evaporation can be demonstrated.

2. A colloidal coat was substituted as the evaporating surface of the porcelain cup.

3. Gelatin, agar agar, gum arabic, tragacanth, Karo syrup and collodion were used as evaporating surfaces.

4. With a relative degree of precaution, heights of mercury exceeding atmospheric pressure can be easily attained.

5. Gelatin seemed to afford the most satisfactory coating. It was easily applied and gave consistently high records. Out of 41 successive trials with 20% gelatin the average total rise of mercury was 129.1 cm. with a minimum of 81.9 cm. and a maximum of 188.7 cm.

6. The highest record, 226.6 cm., was attained by using a 25% gelatin coat.

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SOME RECENT WORK ON THE STRUCTURE OF THE PLANT CELL WALL.*

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The original researches of Nägeli (26, 27) upon the structure of plant cell walls have stimulated discussion and debate, for more than sixty years. Today the Nägeli theory is still under scrutiny and the issue is by no means settled. The problems of plant cell wall structure are no longer exclusively those of the botanist, but in recent years have aroused the interest of physicists, chemists and even of the mineralogists. Direct microscopic observations have been supplemented with extensive studies under polarized light and with the X-ray. It has been only during the past ten or fifteen years that our knowledge of the physical structure of cell walls has advanced materially, beyond the stage at which Nägeli left it, almost three-quarters of a century ago.

It is the purpose of this paper to summarize briefly some of the recent and important work in this field, and to present a picture of plant cell wall structure as recent research has revealed it. No attempt will be made to review the vast amount of literature on the subject prior to the twentieth century, or even that of the early part of this century. Comprehensive and excellent surveys of this literature can be found in the monograph of Wisselingh (34).

The chemical and physical structure of plant cell walls varies widely in the different plant groups and in the same individual. The walls of the bacterial cells, of the algæ and of the mosses are very different from those of the higher plants. In any individual angiosperm there is an almost equally wide range of variation. The walls of root hairs, of epidermal cells, of woody fibers, of bast cells and of other tissues have but few

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characteristics in common. There are, however, two characteristics possessed by most, if not all plant cell walls:

1. Plant cell walls are not chemically homogeneous, but are composed of two or more chemically distinct substances.
2. Plant cell walls are not physically homogeneous, but show stratification, striation and other indications of structural variation.

These facts have long been known and have been the subject of a great deal of study, yet research in the field of cell wall structure has never been more active or promising than at the present time.

One of the most interesting contributions to cell wall research is the work of Hansteen-Cranner (17). This investigator became interested in the toxic effects of Mg, K, and Na ions toward root growth. When a solution containing only one of these cations surrounded the root, all growth ceased. Signs of injury appeared in the region of elongation, i. e., where cell wall growth was most rapid. Further investigation revealed that the toxic effect was the result of changes in the cell wall itself and not the result of injury to the protoplast or nucleus. In solutions of the injurious cations the cell walls became swollen and in some cases burst, revealing the naked protoplast. Mg was most effective, solutions having a concentration of 0.0047% were sufficient to prevent root growth in some cases. Ca, on the other hand, even in solutions of 0.328% produced absolutely no sign of injury. The addition of Ca to Mg solutions not only prevented injury, but permitted normal growth.

It was further found that in dilute solutions of Mg, the roots excreted a cloudy white precipitate. This precipitate was produced by all roots tested and appeared the same in all cases. It contained no trace of protein, but consisted largely of phospholipoids.

In order to determine the origin of this material, the cell walls were freed from the cell contents, with the greatest thoroughness and care. The tissue was macerated and repeatedly washed until microscopic examination showed only cell wall fragments, and microchemical tests for proteins were completely negative. These cell wall fragments freed from every trace of protein material, gave the same precipitate,

indicating that it originates from the cell wall and not from the protoplasm. Hansteen-Cranner therefore concluded that the cell walls in question contained lipoid substances.

Since this original paper, Hansteen-Cranner has greatly extended and amplified his experiments (18, 19, 20), and has conclusively established the fact that the walls of all living plant cells contain relatively large amounts of phosphatides. He has shown that the plasma membranes are also largely, if not entirely, composed of these phosphatides, and that they are in direct and intimate connection with the phosphatides in the cell wall. The plasma membrane is not sharply delimited from the cell wall, but is continuous with the phosphatides that saturate the cell wall. During plasmolysis the plasma membrane does not separate cleanly from the wall, but retains fine thread-like connections with it. This fact, although largely ignored by workers in plasmolytic phenomena, has been previously reported by other investigators (19).

The Ca ion brings about a precipitation of these phosphatides, forming new membranes. The earth alkalies and the mineral acids act in a similar manner. The alkalies and the hydroxides in dilute concentrations act in the opposite way, producing a swelling and a gelatinization of the material. Whenever these ions come into contact with the phosphatides of the cell wall and of the plasma membrane, new membranes are formed—membranes having a widely different character from those normally present.

Hansteen-Cranner has thus demonstrated that the solid cell wall skeleton of cellulose or hemicellulose is permeated with phospholipoids. These phosphatides are not stable compounds, but are easily and extensively modified by changing conditions. Temperature changes, increases or decreases in acidity, the presence or absence of certain ions, and other factors bring about modifications, often extensive modifications, of these phospholipoids in the cell wall and plasma membranes. The formation, for example, of firm insoluble membranes with Ca ions throws much light upon the unfavorable influence that Ca ions exert upon water absorption, and possibly upon the difficulties some plants have in living upon soils rich in Ca. On the other hand, the formation of loose, gelatinous membranes in the presence of K ions may explain the favorable influence of this ion upon water absorption. The fact that phospholipoids bear sugar and probably are the path of sugar

movements from cell to cell, suggests a possible explanation of some of the problems of cell wall growth, or even may explain the idea that the outer layer of the protoplasm (phospholipoid) is itself transformed into cell wall material (1).

Grafe and his co-workers, in a series of papers (13, 14, 15), have confirmed Hansteen-Cranner's work and have carried out extensive investigations upon the chemistry of these phosphatides. From this work it becomes clear that these phosphatides have an important role in cell activities. Not only do the phosphatides determine and control in the large measure the permeability of the cell membranes, but there is also evidence that they and not protein, may form the chromatin of the nucleus and so be related to the whole heredity mechanism.

In a paper published after his death, Hansteen-Cranner (19) went so far as to consider the cell wall alive. Since the wall contains large amounts of phosphatides which are always in intimate union with the outer protoplasmic layers, he thinks that it should be considered a living and physiologically active structure.

This is a view to which few physiologists would subscribe, none-the-less it is an idea that challenges interest. Most botanists have long been accustomed to considering the cell wall as a passive, non-living framework surrounding and supporting the living protoplast. It now appears that no sharp line can be drawn between cell wall and protoplast, but that portions of the latter penetrate more or less completely, the intermicellar spaces of the former. Before the Hansteen-Cranner conception can find general acceptance however, much more must be known about the nature of these phospholipoids, and about their physiological relations with the protoplasm and with the cellulose crystals that form the cell wall skeleton.

Suggestive as this work is, it is the solid skeleton of the cell wall that has attracted the most attention. The physical structure of this framework may be studied in a number of ways:

1. Direct microscopical observation.
2. Microchemically.
3. With polarized light, and with the ultra-violet light.
4. X-ray analysis.

Direct microscopical observation was of course the first method to be used. It revealed the stratification of thick walls and the striation visible on many cell walls. The stratification is more or less distinct in cross section and its visibility varies with the water content of the wall, a fact which has led to the theory that differences in water content *cause* the stratification. The surface markings are of various types: spiral and other striations, cross bands, folds, etc.

The recent work of Aldaba (1) on cell wall structure shows that direct microscopic observation alone, under proper conditions, can give much more information about the structure and development of cell walls than was formerly possible. Aldaba has developed a method of isolating the developing cell intact. This permits a study of the entire cell directly, without resorting to sectioning. Using this method he has shown that some bast fibers are built up of a large number of lamellæ, which seem to arise as a transformation of the outer protoplasmic layer into cell wall material. This observation is of particular interest in connection with the work of Hansteen-Cranner, just discussed.

Microchemical studies have shown the kind, the amount and the location of the different membrane substances in the cell wall. They have shown the chemical changes that occur during the development of the wall, and have furnished a basis for theories of cell wall formation and growth. Wisselingh (34), and more recently Kisser (24), have through the use of microchemical methods shown that cutinized membranes are composed of three distinct lamellæ, an outer layer of pure cutin, an inner layer of pure cellulose, and a middle layer of cutinized cellulose. Wisselingh (34) has also demonstrated that the cork cell wall is likewise composed of three separate lamellæ, an outer layer of strongly lignified cellulose, a thin inner layer of cellulose usually more or less lignified, and between these, a thicker layer of suberin. Anderson (3), also using microchemical methods, has shown that it is possible to separate from one another, the lamellæ composing bast fibers of flax, and to study their structure when so isolated. The same author (4) has applied microchemical methods to a study of collenchyma cell walls and has shown that in such walls there is an alternation of pectic and cellulose lamellæ. The cellulose lamellæ can be separated from the pectic lamellæ and studied separately, for the finer details of their structure. Howe (23),

working on root hairs microchemically, finds a layer of pectic material and a layer of callose forming the wall. Wisselingh (33), Tiffany (32), and Wurdack (35), have shown that the cell walls of various green algæ are also composed of distinct lamellæ, containing one or more different chemical substances. Klein (25) has investigated the cell walls of some blue-green algæ and found them composed of lamellæ, in which the different cell wall constituents were more or less intermingled with one another. Bunning (7) and Shaw (30), found that this type of cell wall structure is present in the Sphagnaceæ and in the fruit coats of *Nelumbo lutea* respectively.

The papers here cited are sufficient to make clear the contribution that microchemistry is making toward solving the problems of cell wall structure. Microchemical studies of the plant cell wall have revealed it as a complex structure composed of distinct lamellæ, the lamellæ in turn being composed of one or more different chemical substances.

The units of cell wall structure, however, are submicroscopic and consequently must be studied in some other way. Most of our information about the fundamental units of cell wall structure has been derived from studies with polarized light and the X-ray.

Cellulose is doubly refractive and this double refraction supplies information regarding the finer details of its structure. As a result of his studies with polarized light, Nägeli considered the cellulose wall as being composed of submicroscopic crystalline micellæ that were definitely arranged in lamellæ. He believed each micellar unit to be surrounded normally with a film of water, and so separated from one another. The stratification visible in cross-section was explained by assuming that the micellæ were of different size and so adsorbed different amounts of water. As the water content of the wall decreased, the micellæ would approach one another, and the stratification would be less apparent. In completely dry cell walls the micellæ would be in direct contact and no stratification would be seen. Microscopical observations showed that the stratification actually does disappear as the wall is dried.

To account for the unequal swelling of cell wall in different directions (i. e., along the longitudinal, tangential and radial axes), Nägeli assumed that the micellæ were elongated. Cubical micellæ would adsorb water equally on all sides and so produce uniform swelling. Cellulose cell walls, however, do not swell

uniformly, but much more in the radial and tangential directions than in the longitudinal. If the units were elongated and arranged parallel to one another in the wall, the amounts of water adsorbed will not be equal on all sides, and therefore the resulting swelling must also be unequal, (Fig. 1).

This theory of water films apparently received support from the results of X-ray investigations which showed that when swelling took place the micellar units themselves did not increase in size, but merely altered their distance relations with one another (31).

Forsaith (9) has suggested an interesting modification of the Nägeli theory to account for the observed behavior of different woods. The checks and fiber ruptures in wood indicate a spiral structure in the cell wall, while the fracture

Fig. 1. Diagram of micellar orientation according to Nägli. (After Frey.)

surface of green wood broken along the grain is longitudinal. The thin-walled vessels split cleanly along their longitudinal axis and the edges of the break are smooth. Forsaith explains this by assuming the break to have occurred in the water films between parallel, longitudinally oriented micellæ. The spiral structure he explains by supposing the micellæ to be of a rhombohedral shape and so arranged that the water films on the ends of the micellæ form spiral lines of weakness (Fig. 2). This structure explains equally well the unequal swelling of wood blocks and the spiral and longitudinal cleaveage planes that occur in wood cells.

Fischer (8) however, has recently pointed out that evidence from both purely physical as well as physiological sides is strongly against the existence of such water films. If two smooth glass plates are pressed together and water is allowed to rise by capillarity between them, they are not pushed apart, but drawn tightly together. Even more conclusive is the fact that alcohol, ether and chloroform which enter the cellulose wall more rapidly than water, produce absolutely no trace of

swelling. Were swelling the result of liquid films around the micellæ, these liquids should produce it. These facts have made necessary a modification of the original Nägeli theory. It is highly probable that the spaces between the micellæ are not empty, as Nägeli believed, but are filled with a highly hydrophilic colloid. The general presence in cell walls of the hydrophilic colloidal phosphotides discovered by Hansteen-Cranner (loc. cit.) is of undoubted importance in this respect. Other workers have suggested other colloidal substances. In young membranes this colloidal intermicellar substance may be of pectic nature. In young Closterium walls one can see the

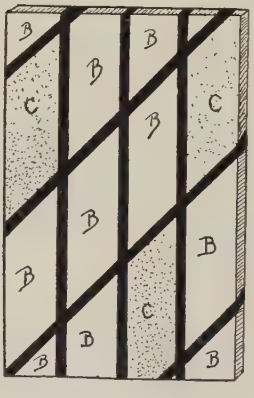

Fig. 2. Forsaith's modification of the Nageli diagram of micellar orientation. Solid black bands represent water films.
B, Cellulose units; C, Lignincomplex. (After Forsaith).

double refraction of the cellulose gradually appear and increase in the isotropic membrane (10). In cellulose walls an amorphous cellulose may form the colloidal matrix (21). In cutinized and suberized membranes, it may be the cutin and suberin (10) and in lignified walls, the lignin. That lignin is *between* the cellulose micellæ and not *in* them is proven by the fact that lignified walls give the X-ray pictures of pure cellulose (10).

The existence of double refraction alone is not sufficient to prove the crystalline nature of cellulose units. Double refraction can also be produced by non-crystalline, isotropic units under certain conditions (2). Ambronn (2) by studying the behavior of cellulose under polarized light, has been able to prove both the existence of micellæ in the cellulose walls and the crystalline character of these micellæ.

If isotropic rods, (glass, for example), whose diameter and distance from one another are small relative to the wave length of light, possess a refractive index of N_1 and are imbedded in a liquid whose refractive index is N_2, the body will appear doubly refractive. If N_2 is varied, the double refraction varies accordingly. When $N_1 = N_2$, there is no double refraction, but when $N_1 \gtrless N_2$ a value arises. The curve of these variations is parabolic in form. By saturating the plant membrane with liquids of increasing refractive index (water, alcohol, glycerine, anilin, etc), Ambronn found his curve agreed with the theoretical curve, thus proving the existence of such submicroscopic rods in the membrane. Since he was never able

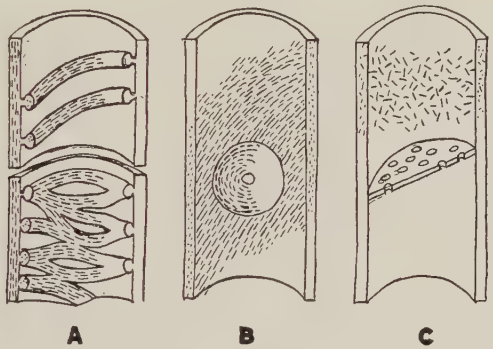

Fig. 3. The orientation of the crystalline micellæ in different cell walls. A, Tracheæ with spiral and reticulate thickenings; B, Tracheid with bordered pit; C, Sieve tube. (After Frey).

to find a value of 0, i. e., his curve did not fall to the 0 point, he concluded that the rods (cellulose micellæ) were themselves doubly refractive (12).

Frey (10, 11, 12) has extended this work in brilliant fashion. He has developed a method of determining the orientation of the micellæ in the cell wall by using a polarizing microscope. Since the morphological long axis of the submicroscopic crystalline micellæ corresponds to the direction of the greatest refractive index, it is not difficult to determine the orientation of the micellæ in the wall. It resolves itself into a matter of determining the direction of the greatest refractive index.

The long axis of these micellæ often parallels the striations visible on the wall surface. In strong cell walls (bast fibers, etc.), the micellæ are united to form long fibrillæ, which are

usually spirally wound around the cell at steep angles. Groups of these fibrillæ can be seen by swelling the cell wall. They have recently been studied by a number of investigators, particularly in the commercial fibers. (Anderson (3), Ball (6), Nodder (28), Reimers (29) and others).

Frey (12) has determined the orientation of the micellæ in a number of different cell walls. The micellæ exhibit a wide variety of patterns and show no constant or definite arrangement common to all cell walls. In bast fibers they are arranged nearly parallel to the long axis of the cell; in sieve tubes and lactic ducts the arrangement is irregular, with no definite orientation; in tracheæ the micellæ parallel more or less the long axis, but in the spiral and reticulate thickenings are nearly perpendicular to the long axis of the cell; in tracheids they are arranged in a steep spiral except at the bordered pits, where they are concentrically arranged around the pore opening. (Fig. 3).

Cutinized membranes such as are usually present in epidermal cells consist of three layers: an outer layer of cutin, an inner layer of cellulose, and between, a layer of cutinized cellulose. This structure has been worked out microchemically (34). Studies with polarized light both confirm and extend this conception. Frey (11) has demonstrated that with polarized light it is possible to determine accurately the location and the degree of cutinization of such membranes. Cutin is optically negative, while cellulose is optically positive. With the insertion of a Gypsum plate Red 1 in the polarizing microscope, these two membrane substances give the contrasting addition and subtraction colors. The cutinized portions of the wall appear a brilliant yellow, while the adjacent cellulose appears bright blue. With the rotation of the stage through 90 degrees the colors are, of course, reversed.

Examination of the walls of heavily cutinized cells (epidermal cell walls of Clivia nobilis and Acuba japonica) under polarized light brings out distinctly the three layers. The outer layer of cutin is isotropic, probably because of the physical arrangement of the micellæ. The two inner layers of cutinized cellulose and cellulose, respectively, are both anisotropic, but the former is optically negative, while the latter is optically positive. The layer of cutinized cellulose shows a region of maximum anisotropism at its center, the degree of double refraction decreasing from the center toward the layer of pure

cutin on the outside and toward the layer of cellulose on the inner side. The cellulose layer, likewise, shows a higher degree of double refraction at its center.

The polarizer further reveals that in some cases (Acuba japonica) the layer of cutinized cellulose is not continuous, but that each epidermal cell has a separate, distinct, plate-like layer of its own. Frey has also shown that the boundaries between these three layers are sharp and distinct, there being no gradual transition from one to the other.

Ultraviolet light passes through cellulose but not through cutin, so photographs taken with ultraviolet light will show whether any cutin is present in the cellulose layer. This is not the case; the cellulose layer shows no sign of cutin even at the extreme outer edge of the layer. The boundary is sharp and definite. No study of the boundary between the cutinized cellulose layer and the outer layer of pure cutin can be made with ultraviolet light, since both appear black. This can be done, however, with polarized light, for the cutin layer is here completely isotropic, because of its physical structure, and the cutinized cellulose layer is anisotropic. Here again, the boundary between the two is sharp and definite, (10).

In this way studies of the membrane with polarized light have extended the results of microchemical investigations, as well as completely confirmed them. The use of polarized light in specific cases offers a much simpler and more precise method of learning the relations between the various cell wall lamellæ, than microchemical methods.

Pectic compounds are isotropic and can be detected in cell walls when relatively unmixed with cellulose, by the use of polarized light, (11). When pectic compounds occur associated with cellulose, the double refraction of the latter obscures the isotropic character of the former, (if the cellulose is present in appreciable amounts), and the presence of the pectic material cannot be clearly detected (4).

Hemicelluloses are at least in part doubly refractive, an indication that like pure cellulose, they are probably composed of crystalline micellæ (11).

Polarized light also gives evidence as to the relations of the lignin complex to cellulose. Lignified tissue is doubly refractive, but if the cellulose is removed, becomes completely isotropic (11). This indicates that the lignin complex is amorphous and that the anisotropic character of lignified walls is due to the

cellulose present. The removal of the lignin complex from lignified tissue does not affect in any way the degree of double refraction. The amorphous character of the lignin is also confirmed by X-ray analyses as has been mentioned previously.

In brief, polarized light offers not only a method of detecting and accurately localizing certain membrane constituents, but makes possible a determination of the orientation of the crystalline micellæ in the cellulose cell wall. It has been responsible for a great increase in our knowledge of the details, particularly the submicroscopic details, of cell wall structure. In speaking of the importance of polarized light, in relation to the evidence for the Nägeli micellar theory, Frey (10) says,

"Die hier summarisch aufgefuhrten Grunde, die für die Existenz der Micelle sprechen, und die Tatsachen die uber das Auftreten und die Eigenschaften der Intermicellarräume Auskunft geben, zeigen deutlich dass die Micellartheorie nicht mehr hypothetisch, sondern weitgehend bewiesen ist."

X-ray analyses have made possible a still further advance in solving the problems of cell wall structure. X-ray pictures of cellulose fibers give the interference patterns of crystal structure. The character of these interference diagrams indicates the arrangement of the crystalline units in the wall. Plant fibers have generally shown a crystalline fibrillar structure in which the crystals lie with their morphological long axis in approximately the fiber axis. The orientation of these fibrillæ varies in different fibers. In bast fibers of flax the fibrillæ run in a steep spiral, but the direction of the spiral alternates in successive lamellæ of the wall. In bast fibers of ramie and hemp, the fibrillæ also have a steep spiral arrangement, but the angle of the spiral varies in successive lamellæ. Cotton fibers show the same spiral structure, but here the spiral reverses its direction at points in the same lamella. The interpretations of X-ray photographs of the fibers agrees with these direct microscopical observations. Cotton, for example, gives an interference pattern that indicates a structure of spirally wound fibrillæ, while the diagram of flax fiber indicates a reversal of the fibrillar direction, (21).

Beyond confirming the presence of definite crystalline units in the cellulose wall, and indicating the arrangement of these crystals, the X-ray analyses have shown that an amorphous material is also present. To quote Herzog, (22),

"Aus den Röntgen-Bildern der Fasern ergibt sich die Anwesenheit nicht unerheblicher Mengen amorpher Stoffe—vielleicht auch amorpher Cellulose—neben den Krystallen. Man wird sie als Einbettungs—substance ansehen."

That cellulose is not a single chemical compound has also been suggested by Arrhenius (5) from studies of its reaction energy. Haller (16) came to the same conclusion from investigations on the behavior of the hydro- and oxycelluloses, derived from cotton fibers, when treated with NaOH.

This amorphous substance surrounding the crystalline units acts as an insulating material by preventing the crystalline micellæ from coming into direct contact with each other. Were no such substance present, the continuous stresses produced in the cell wall by such external forces as winds, would bring about a recrystallization, comparable to that occurring in steel under continuous hammering. Such a recrystallization would greatly reduce the resistance of the cell wall to such stresses. The presence of this amorphous, intermicellar, insulatory, colloid prevents this from taking place (21).

Herzog (21) suggests an interesting theory to account for the orientation of the crystalline micellæ in plant fibers. The first wall formed by the cell is amorphous (pectic material). In this amorphous substance the cellulose mother substance is assumed to be present as small droplets. These droplets rapidly crystallize. Since, however, they are under stresses brought about by the growth of the cell wall, they crystallize in definite directions. The long axis of the crystal conforms to the direction of the stresses, i. e., they are arranged more or less parallel to the long axis of the cell. The degree of tension determines the degree of crystallization. Such crystallization occurs in the manufacture of artificial fibers from cellulose solutions, and it is entirely possible that a similar crystallization takes place in the formation of the cell walls of fiber cells.

This cannot be the only factor involved, however, for the direction of these crystalline fibrillæ is not constant in all fibers, (flax, hemp, ramie, etc.). Such a theory could hardly account for the reversal of the direction of the spiral in bast fibers of flax, for example. It is possible that cellulose crystals in this case are isomeric, and that the alternate left and right hand spirals is the result of an alternation of isomeric celluloses (3).

The theory of Herzog also runs into difficulties when the case of sieve tubes and lactic ducts is considered. In these cell

walls, as Frey (12) has shown, the micellæ are irregularly distributed with no definite arrangement. These walls must also have been subject to the stresses of cell wall growth, yet here these forces have apparently produced no definite micellar orientation.

X-ray studies of cellulose walls confirm both the results of microchemical work, and the results of polarized light investigations. These three methods of study have established the crystalline micellar theory of cell wall structure upon an impregnable basis.

Summary.

In the light of modern research, all cellulose cell walls, as well as those containing cellulose in considerable amounts, possess a submicroscopic structure of crystalline micellæ. These micellæ are imbedded in an amorphous matrix, the chemical nature of which varies in different walls and probably at different stages in the development of the same wall. The orientation of the crystalline micellæ is not constant in all walls, but varies widely.

From the standpoint of microscopic structure, the wall is a complex of several chemically different lamellæ. The lamellæ may consist of but one chemical substance, and alternate with lamellæ of other cell wall constituents, or the various constituents may be present in the same lamella.

This complex structure is saturated with colloidal phosphatides, that are in intimate union with the outer protoplasmic layers. These colloidal phosphatides are unstable and are readily modified by varying conditions. The physiological significance of their presence in the wall is not at the present time completely understood.

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SPERMATOGENESIS IN BRANCHIPUS VERNALIS.

PART III.

SECONDARY SPERMATOCYTE, SPERMATID AND SPERMATOZOÖN.

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In two previous papers, Baker and Rosof, Part I, 1927 and Part II, 1928, the general description of the testis and the behavior of the chromatin of the spermatogonia and primary spermatocyte in Branchipus vernalis was described. The outstanding features of the behavior of the chromatin in the spermatogonia are as follows: (1) There are twenty-three chro mosomes in the spermatogonia of Branchipus vernalis: (2) There are eleven pairs of homologous chromosomes and one accessory; (3) In the late prophase stage of the spermatogonia, some homologous chromosomes are paired. This pairing was interpreted as being more of a chance occurrence than a regular behavior in the activity of the chromosomes.

The following summary briefly states the changes occurring in the chromatin of the primary spermatocyte:

(1.) The early primary spermatocyte is characterized by a reticulum in which the arrangements of the chromatin is in the form of masses and strands. There is a difference in the number, size, form and relationship of these chromatin aggregates and strands. Some of the chromatin bodies found in different nuclei show marked similarities. The reticulum characteristic of this early stage is affected by the separation of chromosomes from the dense mass of the telophase plate of the last spermatogonial division. This is the early prochromosomal stage;

(2.) The reticulum becomes less pronounced and the chromatin bodies reveal characteristic shapes. The number of chromatin masses is not constant in the various nuclei but in no case does the number of masses exceed twenty-three. This is the late prochromosomal stage;

(3.) The recticulum is less pronounced. In the previous stage, the chromatin aggregates were the more conspicuous elements in the nucleus, whereas, in this stage the strands are

more pronounced. Some of the strands resemble the elongate and ill-defined chromosomes. This characterizes the unraveling stage. These three stages compose the preleptotene stage;

(4.) The chromosomes now undergo a series of changes involving the following processes: (a) Synapsis of homologous chromosomes; (b) Orientation of unlike chromosomes into an end to end association; (c) Equalization in thickness and change in form of chromosomes into definite, uniform and elongate chromosomes. The simultaneous manifestation of these three processes is interfered with by the differences in the relationship of the chromosomes as they emerge from the last spermatogonial telophase. This constitutes the leptotene stage;

(5.) The chromosomes for the most part are well defined bivalent loops which are associated end to end, and are peripherally arranged in respect to the nucleus. This marks the close of the processes which are operating in the pre-leptotene and leptotene stages and marks the initiation of the processes which are involved in the post-leptotene stage. This is the transitional stage.

The post-leptotene stages following the transitional are summarized as follows:

(6.) The chromosomes may or may not form bouquets prior to the following pachytene stage. These two methods of chromosomal transformations may be interrupted by the intervention of synizesis;

(7.) The chromosomes shorten and assume characteristic forms. These chromosomes are scattered throughout the nucleus. This is the pachytene stage;

(8.) The chromosomes elongate and thicken and simulate an ill defined bouquet. Some of the chromosomes are partially coalesced and are less distinct. This constitutes the second orientation;

(9.) Chromosomes become more diffuse and separate, but at the same time the individual chromosomes are again undergoing a process of shortening. This corresponds to the diffuse stage;

(10.) Chromosomes are shortened and contracted and are so intermingled that they somewhat simulate synizesis. This is the second contraction;

(11.) Chromosomes now are no longer diffuse but are separated and again assume characteristic shapes. This is the diakinesis stage;

(12.) The chromosomes become still further contracted and rounded. At this time some of the chromosomes are joined to each other. The nuclear membrane disappears. This constitutes the late prophase stage in contrast with all previous stages which are parts of the early prophase.

(13.) Metaphase follows and meiotic division ensues.

In regard to the question of synapse it is inferred that the interval and mode of synapse is dependent upon the arrangement of chromosomes as they emerge from the last spermatongonial division and to the sequence of the processes as indicated in the leptotene stage. The chromosomes of this species do not synapse in the same condition nor do they synapse at the same time. If the chromosomes synapse while in the form of filaments, then the mode of synapse is unquestionably parasynaptic. On the other hand, if synapse occurs during the clumped condition of chromatin, it is impossible to determine with certainity whether or not the mode of conjugation is parasynaptic or telosynaptic.

The present paper is limited chiefly to the description of the observations covering the behavior of chromosomes of the secondary spermatocyte, spermatid and spermatozoön.

THE SECONDARY SPERMATOCYTE.

The secondary spermatocytes are arranged in the testis in cysts as were the spermatocytes of the first order. In transverse section of the testis the cysts are well defined and are distinctly localized toward or near the immediate vicinity of the lumen of the gland. The cells composing these cysts are comparable to the cells of the first order only in their shape which is round or oval unless encroached upon by surrounding cells and under such conditions a variety of cell contours may result. The secondary spermatocytes are somewhat smaller than the primary spermatocytes.

Following the primary spermatocyte division, the nucleus of the secondary spermatocyte reveals the chromatin disposed in a darkly staining and clumped mass in which the individuality of the chromosomes are more or less obscured. This condition is shown in Figure 1.

The ensuing changes that the chromosomes of the secondary spermatocyte undergo are unlike the changes which occur in the early prophase of the primary spermatocyte. The behavior

of the chromosomes of the secondary spermatocyte is uncomplicated in that no factors are involved excepting those which operate in connection with mitotic divisions.

Figures 2 and 3 show the chromosomes less intimately related. The individual chromosomes can now be distinguished by their differences mainly in size, and to a lesser extent by differences in their morphological aspects. Some of the chromosomes show connecting strands which are similar to those which connect metaphase chromosomes of the primary spermatocyte. This feature is more pronounced in Figure 2 than in Figure 3. These two figures contain eleven chromosomes each.

Further changes result in the elongation and change in form of the chromosomes so that each chromosome is morphologically dissimilar to the others, and at the same time the connecting strands are less pronounced and for the most part disappear. The chromosomes now are more or less uniformly distributed throughout the nucleus and are somewhat peripherally arranged in relation to the nuclear membrane, (Figure 4.). The full count of chromosomes is not present in Figure 4 since only ten are visible. The chromosomes in Figure 5 are in a later stage and the chromosomes are more elongate. In the lower portion of the nucleus, two strands connecting chromosomes are seen. Some of these nuclei have their central portions somewhat constricted. This cell gives a count of twelve chromosomes.

The next change reveals the chromosomes undergoing contraction. The individuality of the chromosomes at this stage is most pronounced and they exhibit in the main well defined and characteristic contours. It may be emphasized that the chromosomes of this stage are comparable to the spermatogonial chromosomes of a similar stage. Strands connecting chromosomes are no longer visible, (Figures 6, 7 and 8). Figures 6 and 7 show eleven chromosomes while Figure 8 has twelve.

Figures 9, 10 and 11 are polar views of metaphase. These chromosomes are in the form of rounded bodies which may be distinguished from each other by differences in size. Figure 9 shows a central mass of chromosomes which are closely approximated from which extend spindle fibers. There are five chromosomes in the immediate vicinity of this central mass which are apparently lagging behind. No nuclear membrane is present in this or in the succeeding figures of the secondary spermatocyte.

Twelve chromosomes are seen in Figure 10. The arrangement of the chromosomes as seen in this figure is quite character-

istic of secondary spermatocytes of this stage. Figure 11 shows a mass of chromosomes with one chromosome free. It is inferred that this is the accessory chromosome.

Figures 12 to 14 are profile views of early metaphase. The chromosomes of Figure 12 show a slight irregularity in their behavior in that three chromosomes are proceeding to their respective poles in advance of the remaining chromosomes.

Figure 13 shows a dense metaphase plate which is so orientated that it reveals an oblique view. The accessory chromosome is present and is proceeding precociously and undivided to one pole. Figure 14 presents a similar view, however, the accessory chromosome is divided and occupies the poles of the spindle.

Figure 15 is an anaphase while Figure 16 and 17 are telophases. The latter figure is of interest in that it shows the beginning of a nuclear membrane. The contained chromosomes do not show the massed condition which is more in evidence in the previous stages.

THE SPERMATID AND SPERMATOZOÖN.

The spermatids are located around or near the lumen of the testis in a cyst-like arrangement being present throughout the entire extent of the testis but far more numerous in the anterior extremity of the gland. The arrangement in the cysts is not as typical as was the condition in the preceding stages and the contour of the cyst is not so well defined and is frequently absent. Spermatids may be found also in the lumen of the testis intermingled with the spermatozoa.

In shape, the spermatids vary from a round or oval to an oblong form and are distinctly smaller in size than the spermatocytes of the second order.

Figure 17 shows a late telophase stage of the secondary spermatocyte and reveals the characteristic massing of the chromosomes. In some cells this packed condition partially obscures the individual chromosomes, but usually the chromosomes can be distinguished. Eleven chromosomes are seen in Figure 18. These chromosomes are rounded and somewhat massed. Strands connecting chromosomes are visible. There is little difference in shape of individual chromosomes but a difference in size is more pronounced and with careful scrutiny the individuality of the chromosomes can be detected. The

area occupied by the chromosomes is marked off from the cytoplasm by a nuclear membrane. On the left, in the immediate proximity of the nuclear membrane are two well defined centrosomes. This condition of divided centrosomes is common in spermatids and spermatozoa. The cytoplasm in this figure as well as in the succeeding figures is not shown. This is the typical condition of the nucleus of a spermatid following the secondary spermatocyte division.

Following the rounded condition of the chromosomes, they assume a more elongate form with characteristic shapes. The strands connecting the chromosomes are more evident, some being more conspicuous than others. Figure 19 shows the chromosomes for the most part in a rounded condition. In the more central portion of this nucleus, an elongate chromosome is seen from which several strands extend to neighboring chromosomes. There are twelve chromosomes in this nucleus.

The chromosomes in Figure 20 show a more pronounced change in form than does the previous figure. A few connecting strands are present and some of the chromosomes are enlarged. Some of these chromosomes appear to be joining with other chromosomes. Although the full number of chromosomes is present in this nucleus a count of only ten chromosomes is obtained.

The condition of the chromosomes in figures previously described indicates the initiation of the changes which results in the final configuration of the spermatozoa. These changes involve the rearrangement and coalescence of chromosomes and results in an apparent diminution of the number of chromosomes in the spermatozoa. Figures 21 to 29 inclusive show the configuration which the chromosomes undergo in the early stages of this coalescence of unlike chromosomes.

It is difficult to obtain an accurate count of chromosomes in Figure 21 on account of the coalescence of some of the chromosomes. However, nine distinct bodies are visible. For the most part, the chromosomes reveal slight differences in shape which is indicative of the fact that the chromosomes have a tendency to change their form, but this change is not so pronounced in most cases as to obscure the recognition of the individual chromosomes. In some cases even though the chromosomes are partially coalesced certain features still remain which assure the identification of the chromosomes

entering into the coalescence. This figure also shows two well defined centrosomes.

Figure 22 shows eight bodies of chromatin six of which are well defined. The two chromosomes in the central part of the nucleus are partially coalesced. Strands connecting chromosomes are evident. Some of the chromatin bodies of this nucleus resemble the chromosomes of previous stages in the spermatogonia, primary and secondary spermatocytes, as well as, chromatin bodies in other spermatids. Eight well defined chromatin bodies, some of which are joined by connecting strands are seen in Figure 23. One centrosome is shown in this figure. Six chromatin bodies, five of which are well rounded and peripherally arranged are seen in Figure 24. The more central, larger, elongate and irregularly shaped chromatin body which is connected to three of the peripherally arranged chromatin bodies by single thin strands is the result of the coalescence of several chromosomes. This cell shows one large centrosome.

Figure 25 shows several chromosomes coalescing at the same time, there being only two chromatin bodies which are free. This condition is slightly different from the nucleus just described in that the chromosomes are so orientated as to permit a more rapid union than is indicated in the previous figures. It will be noted that the connecting strands are heavy and that the chromosomes joined by these strands are in close proximity to each other. One centrosome is present.

Figure 26 shows seven chromatin bodies whose appearance is similar to Figures 22 to 24. Two centrosomes are seen in Figure 26. Figure 27 contains seven bodies of chromatin. This figure is different from the preceding ones in that the strands are more numerous.

Figure 28 shows in a pronounced manner the coalescence of chromosomes. One chromatin body is free. Three well defined and rounded chromatin bodies are seen in Figure 29, as well as two large irregularly shaped masses of chromatin. The nuclear membrane is indistinct.

It is evident in the previously described Figures 18 to 29 inclusive that the following processes are involved in the behavior of chromatin: (1) Change in form; (2) Rearrangement; (3) Coalescence. These three processes operate simultaneously, and continue until the final configuration of the chromatin in the spermatozoa is reached. The changes in the morphology

of individual chromosomes do not affect all chromosomes equally and as a result the same chromosomes may be subject to a variation of contour. Nevertheless, with careful observation individual chromosomes can be recognized regardless of the variation that they undergo in the various nuclei even though they have changed in contour they retain sufficient characteristics of size and shape to be distinguished.

The rearrangement of chromosomes is indicated by change in relation that individual chromosomes assume to each other and this relation becomes more conspicuous in the ensuing figures. Furthermore, the picture which results from the rearrangement of the chromosomes of any stage indicates that there are several distinct methods involved in the rearrangement of chromosomes prior to the final configuration in the spermatozoön bodies. The same chromosomes may form a different pattern in different nuclei in the same stage depending upon the various relationships assumed by these chromosomes.

The coalescence of chromosomes is revealed by the apparent reduction in the number of chromosomes without an apparent diminution of chromatin content as the spermatids differentiate. During this process of change in the spermatid there is shown a fusion of chromosomes. The rapidity with which chromosomes fuse is for the most part dependent upon the conditions of the chromosomes, and, as well the orientation of the chromosomes. In the early part of this process when only a few of the chromosomes are coalesced, the chromosomes which enter into this fusion retain sufficient characteristics to make them recognizable and furthermore with careful observation the exact method of fusion of chromosomes can be determined.

Figure 30 shows seven masses of chromatin six of which are partially coalesced. Their arrangement is in the form of an incomplete circle and the individual contours of each mass is discernible. The nuclear membrane is absent.

There are six rounded and well defined chromatin bodies in Figure 31. Their disposition is similar to that shown in the preceding figure but they do not show the degree of coalescence that is indicated in the previous figure. Strands are visible which connect the chromosomes, some of which are heavier than others.

Figures 32 and 33 are similar in that each figure presents five chromatin bodies which are connected to each other by varying sized strands. The arrangement of these chromatin

bodies simulates a quadrangular form, the angles of which are occupied by a chromatin body. The other chromatin body partially obscures this configuration. Figure 33 shows the entire spermatid.

Figures 34 to 40 inclusive show the same number and similar orientation of chromatin bodies. The arrangement of the chromatin in these cells simulates an irregular quadrangle with a chromatin body at each angle. These bodies are connected by communicating strands which form the sides of the figure. The patterns presented by these chromatin bodies differ from each other in the following respects: (1) difference in size and form of the chromatin bodies; (2) difference in the length and degree in attenuation of the connecting strands. An additional strand is present in Figure 34 which connects two chromatin bodies. No accessory chromosome is present in Figures 35 to 40.

These figures just described suggest the manner in which the final spermatozoön configurations of chromatin bodies may be achieved. It may be inferred that the connecting strands which are thin and attenuated will disappear while the heavier one will persist and the smaller chromatin bodies will coalesce with the larger bodies. This inference is supported by the succeeding figures.

Figures 41 to 43 show four chromatin bodies which are similar to the figures just described but differ to the extent that the reduction in the number of connecting strands is evident. No accessory chromosome is present. Figure 42 shows the entire cell.

Since the change from a spermatid to a spermatozoön involves a process of metamorphosis, it is impossible to tell just where a line demarcating the differentiation should be drawn. However, the following may be used as a criteria for the identification of a spermatozoön: (1) The cells that contain two or three bodies of chromatin completely or incompletely connected by strands. No accessory chromosome present, (Figures 45 to 62); (2) The cells that contain four bodies of chromatin incompletely connected by strands. No accessory chromosome present, (Figures 41 to 43). The accessory chromosome when present constitutes an additional body in the above mentioned spermatozoa and it can be distinguished by its isolation and shape, (Figures 63 to 71).

Figures 44 to 52 are spermatozoa containing three chromatin bodies. No accessory chromosome is present. Figures 44 and

45 differ from the other figures to the extent that the chromatin bodies are completely connected by strands. One additional strand is present in Figure 44. A complete cell is shown in Figure 44. Figures 46 to 48 are similar stages showing heavy strands which partially obscure the identity of the chromatin masses. Figures 49 to 51 show a similar pattern of chromatin. The chromatin bodies are approximately the same size and shape and the more peripherally placed bodies are connected to the central bodies by a u-shaped strand, Figure 52.

Figures 53 to 62 show the final configuration of the chromatin in the spermatozoa. No accessory chromosome is present in any of these figures. In all these figures there are only two bodies of chromatin. In Figure 53 the bodies are approximately of the same size and the connecting strand is short and thick. Extending from one mass there is a thick extension of chromatin. Figure 54 shows two bodies of chromatin, one being V-shaped and the other elongate. These two chromatin bodies are practically united without the intervention of a connecting strand. The chromatin in Figure 55 is in the form of a crescent. Figures 56 to 58 reveal approximately equally sized masses of chromatin connected by strands which show differences in length, thickness and disposition. Figures 59 and 60 show an elongate mass of chromatin connected by strands to smaller rounded bodies of chromatin. Two unequal sized bodies of chromatin are seen in Figures 61 and 62. In Figure 61, the bodies are connected by a very thin and long strand while the chromatin bodies of Figure 62 are separate. The smaller body of this figure shows an irregular strand extending from it. This figure also shows two well defined centrosomes.

It must not be inferred that the arrangement of the chromosomes in the chromatin bodies is the same in every spermatozoön, as has been previously stated there are several methods by which these various configurations may be produced. The spermatozoa thus far described contain no accessory chromosome.

Figures 63 to 71 are stages of spermatozoa which show, in addition to the ordinary chromatin bodies, an accessory chromosome. Approximately one half of the spermatozoa show the presence of the accessory chromosome. Four masses of chromatin and in addition the accessory chromosome are shown in Figure 63. Figure 64 shows three bodies of chromatin circularly disposed and in addition the accessory chromosome. Three well defined rounded bodies of chromatin which are con-

nected to each other are seen in Figure 65. The accessory chromosome is seen to one side. Figures 66 to 71 show the final chromosomal configuration of spermatozoa that contain the accessory chromosome and it is separate from the main mass of chromatin. By reference to previous figures of mature spermatozoa, (Figures 53 to 62), it is seen that the configuration of the chromatin corresponds in these figures to a similar configuration containing the accessory chromosome, (Figures 66 to 71).

In making a survey of the cysts which contain spermatozoa, it is seen that approximately one half of the contained cells show the distribution of chromatin as indicated by Figures 66 to 71. The shape of the accessory chromosome is quite constant and invariably is isolated from the ordinary mass or masses of chromatin. Approximately, the other one half of the spermatozoa do not reveal an accessory chromosome, (Figures 53 to 62). For this reason, it seems justifiable, therefore, to conclude that in the spermatozoa of Branchipus vernalis sexual dimorphism is clearly demonstrable.

CONCLUSIONS.

1. The secondary spermatocytes are of two types: first, those with eleven autosomes and, second, those with eleven autosomes and one accessory chromosome.

2. The accessory chromosome may divide or proceed undivided to one pole of the cell in the secondary spermatocyte division. In either case the accessory chromosome precedes the autosomes.

3. In the spermatids and spermatozoa the chromosomes undergo a series of changes involving the following processes: (1) change in form of the chromosomes, (2) the rearrangement of the chromosomes, (3) coalescence of chromosomes. These three processes operate simultaneously and continue until the final configuration of the chromatin bodies in the spermatozoa is attained.

4. The arrangement of the chromosomes in the chromatin bodies of the spermatozoa is not the same in every case.

5. The centrosome may or may not be divided in the spermatozoa.

6. Sexual dimorphism is demonstrable in the spermatozoa since approximately one half of the spermatozoa contain the accessory chromosome.

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EXPLANATION OF PLATE I.

(All figures were drawn by aid of the camera lucida at a magnification of 1250 diameters).

Fig. 1. A secondary spermatocyte immediately following the primary spermatocyte division. The chromatin is disposed in a darkly staining mass.

Figs. 2 and 3 are early prophase stages of the secondary spermatocyte showing definite and rounded chromosomes. Each cell contains eleven chromosomes, some of which are connected by intervening strands.

Fig. 4. The beginning of a change in form of the chromosomes is seen in this figure. The connecting strands are less pronounced. This cell does not contain the full complement of chromosomes.

Fig. 5 shows a later prophase stage characterized by the elongation of the chromosomes. Twelve chromosomes are present in this nucleus.

Figs. 6, 7 and 8 show the chromosomes undergoing contraction. These chromosomes are well defined and exhibit characteristic contours. The nuclei in Figures 6 and 7 contain eleven chromosomes, while Figure 8 has twelve.

Figs. 9, 10 and 11 are polar views of metaphase. The nuclear membrane is absent. In Figure 10, twelve chromosomes are seen. Figure 11 shows one free chromosome at the side of the main mass of closely clumped chromosomes and is interpreted as being the accessory.

Fig. 12. A profile view of an early metaphase.

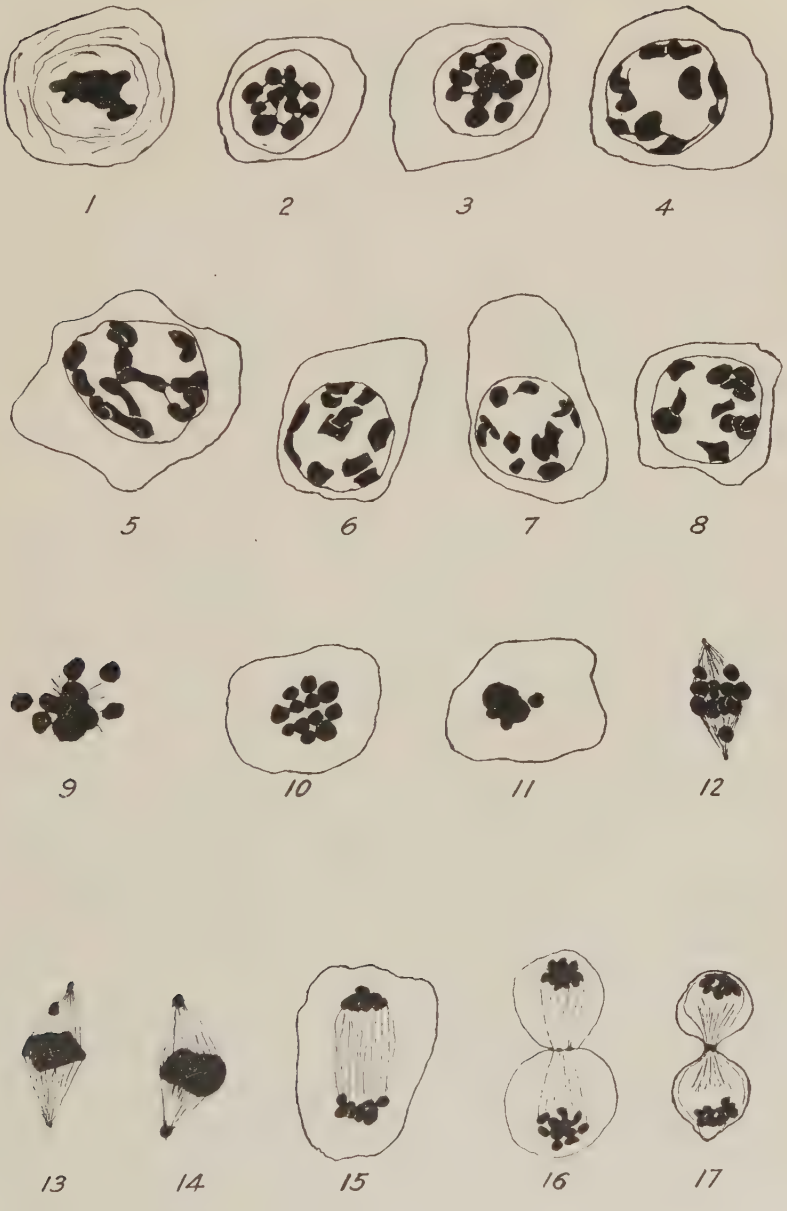
Fig. 13. A profile view of an early metaphase plate oriented obliquely. The accessory chromosome is seen toward the upper pole.

Fig. 14. A profile view of a metaphase similar to the preceding figure. The accessory chromosome is divided and occupies the poles of the spindle.

Fig. 15. Anaphase.

Fig. 16. Telophase. In the lower portion of the figure, eleven chromosomes are seen.

Fig. 17. Telophase. Individual chromosomes are evident. Note the beginning of a nuclear membrane.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EXPLANATION OF PLATE II.

Fig. 18. An early spermatid showing eleven rounded chromosomes. Strands connecting some of the chromosomes are visible. To the left near the nuclear membrane two centrosomes are present.

Fig. 19. This spermatid nucleus contains twelve chromosomes, the majority of which present a rounded contour. The connecting strands are more pronounced.

Fig. 20. A change in form of the chromosomes is evident. Note the apparent joining of chromosomes, and decrease in number, although the full complement of chromosomes is present only a count of ten is obtained.

Figs. 21 to 29 show the configurations which the chromosomes undergo in the early stages of coalescence of unlike chromosomes. It is impossible to make an accurate count of chromosomes in Figure 21, however, nine distinct chromosomes are visible. Two centrosomes are present.

Figs. 22 shows eight bodies of chromatin, six of which are well defined. In the central part of the nucleus two chromosomes are partially coalesced. Strands connecting the chromosomes are pronounced.

Fig. 23. Eight well defined chromatin bodies, some of which are joined by strands, are seen in this nucleus. One centrosome which lies close to the nuclear membrane is visible.

Fig. 24 contains six chromatin bodies. Five of these bodies are round and are peripherally placed. Note the more central, large and irregularly shaped chromatin body which was formed by the coalescence of several chromosomes. One large centrosome is present.

Fig. 25 shows the simultaneous coalesence of several chromosomes. Only two chromatin bodies are free. One centrosome is visible.

Fig. 26. Seven chromatin bodies are seen in this nucleus and also two centrosomes.

Fig. 27 contains seven bodies of chromatin. The connecting strands are more numerous than in the preceding figures.

Fig. 28 shows distinctly the coalescence of the chromosomes. One chromatin body is free.

Fig. 29. This nucleus shows five large chromatin masses, three of which are rounded. The nuclear membrane is becoming less distinct.

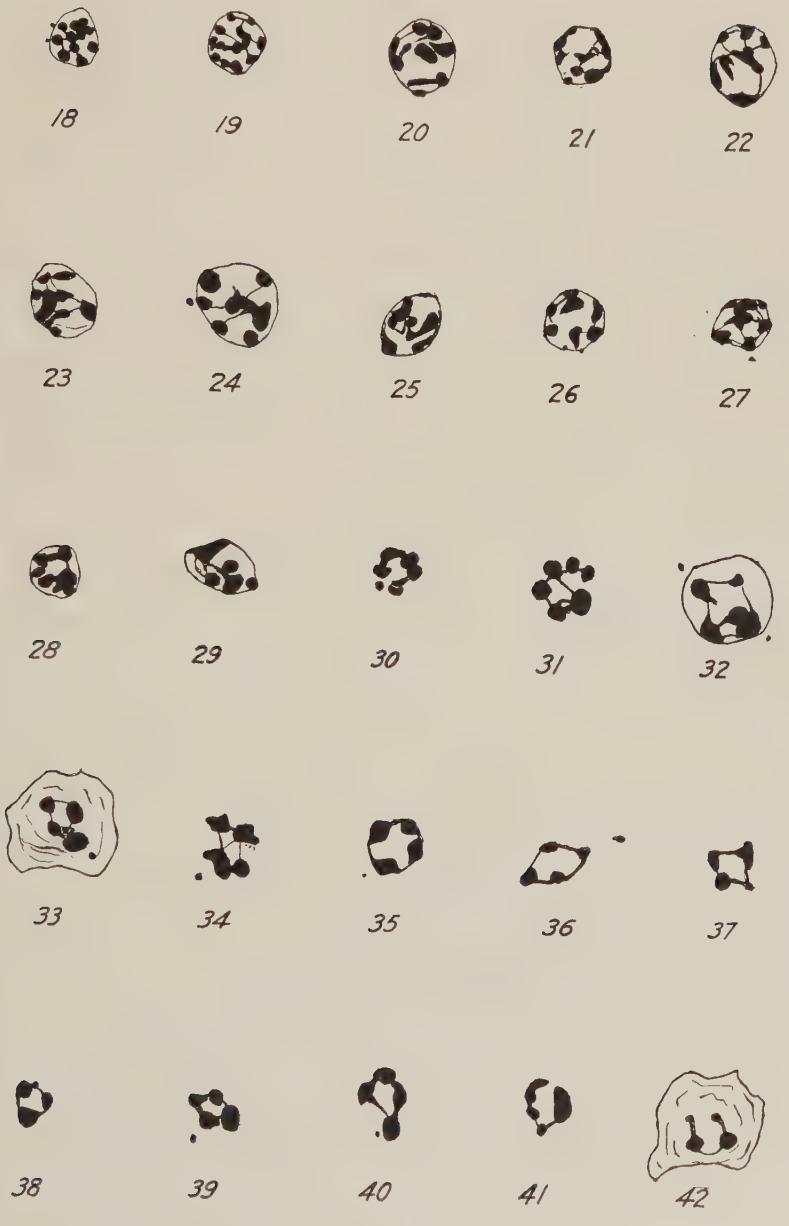
Fig. 30 shows seven masses of chromatin, six of which are partially coalesced, arranged in the form of an incomplete circle.

Fig. 31. Six definite rounded chromatin masses are seen in this nucleus. The degree of coalescence is not so marked. The connecting strands are quite conspicuous.

Figs. 32 and 33 are similar in form. Each of these nuclei present five chromatin bodies connected to each other by varying sized strands. The entire spermatid is shown in Figure 33.

Figs 34 to 40 inclusive. These nuclei show the same number and similar disposition of the chromatin whose arrangement simulates an ill-shaped quadrangle with a chromatin body at each angle. An additional connecting strand is seen in Figure 34. No accessory chromosome is present in these nuclei.

Figs. 41 and 42 show four chromatin bodies, the disposition of which is similar to the preceding figures. A reduction in the number of connecting strands is seen. The accessory chromosome is absent. The entire cell is shown in Figure 42.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EXPLANATION OF PLATE III.

Fig. 43. A spermatozoön containing four chromatin masses. In this stage the accessory chromosome may constitute a fifth body. See Figure 63.

Figs. 44 and 45 are spermatozoa containing three chromatin bodies completely connected by strands. Figure 44 which is a complete cell shows one additional strand. The accessory chromosome is absent.

Figs. 46, 47 and 48 are similar stages of spermatozoa. Heavy strands are visible which connect and partially obscure some of the chromatin masses. The accessory chromosome is absent.

Figs. 49, 50 and 51 are similar in the respect to the number and distribution of the chromatin bodies. Figure 51 is the entire cell.

Fig. 52. The entire cell is shown containing three chromatin bodies of nearly equal size and similar shape connected by U-shaped strands.

Figs. 53 to 62 inclusive show the final configuration of the chromatin in the spermatozoa. Two chromatin bodies are present in these figures and are connected by heavy strands with the exception of Figure 62. This figure contains two centrosomes. The chromatin bodies in Figures 53 and 54 are almost united. The accessory chromosome is absent.

Figs. 63 to 71 are stages of the spermatozoa and show in addition to the ordinary masses of chromatin an accessory chromosome.

Fig. 63 shows four masses of chromatin and an accessory chromosome. The accessory chromosome in the following figures is marked X.

Fig. 64. The entire cell is represented. The chromatin bodies are circularly arranged. The accessory chromosome is seen in the upper part of the figure and is free from the main masses.

Fig. 65. Three rounded chromatin masses are present and are connected to each other. The accessory chromosome is completely isolated.

Figs. 66 to 71 show the final stages of the spermatozoa that contain the accessory chromosomes.

Fig. 66 shows the entire spermatozoön. Two large, irregular and connected chromatin masses are present. The accessory chromosome occupies a position to the right.

Fig. 67. The ordinary chromatin masses are connected by thin strands. The accessory chromosome is seen in the upper part of the figure.

Fig. 68. The accessory chromosome occupies a position above the main mass of chromatin.

Fig. 69. The two ordinary masses of chromatin are connected. The accessory chromosome is seen to the left near a centrosome.

Figs. 70 and 71. The former figure shows two main masses of chromatin with the accessory above and to the right. The latter figure reveals the shape of the entire spermatzoön. The three ordinary chromatin masses are closely approximated, the accessory chromosome occupies an upper position with its convexity pointing downward.

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